BASF
Abteilung Toxikologie
Department of Toxicology

D-67056 Ludwigs-
hafen, FRG

en-db/0201

NOV 17 1994

REPORT

IN VITRO CHROMOSOME ABERRATION ASSAY

WITH

Uvinul T 150

IN V79 CELLS

Project No.: 32M0246/934164

Testing facility:

BASF Aktiengesellschaft
Department of Toxicology, Z 470
D-67056 Ludwigshafen/Rh., FRG

Head of Department of
Toxicology:

Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

Project No.: 32M0246/934164

GLP Statement

Title: Report: In vitro Chromosome Aberration Assay with Uvinul T 150 in V79 Cells

This study was conducted in accordance with the GLP provisions of the "Chemikaliengesetz" ("Chemicals Act"; Bundesgesetzblatt, Teil I, 22.03.90) and with the "OECD Principles of Good Laboratory Practice" (Paris, 1981).

Nov 9, 1994
.....
Dr.rer.nat. H.D. Hoffmann
(Head of Section)

Engelhardt, Nov. 8, 1994
.....
Dr.rer.nat. G. Engelhardt
(Study Director)

Project No.: 32M0246/934164

STATEMENT

OF THE QUALITY ASSURANCE UNIT

Number of test substance: 93/246

Name of test substance: Uvinul T 150

Title: Report: In vitro Chromosome Aberration Assay with Uvinul T 150 in V79 Cells

The Quality Assurance Unit inspected the study, audited the final report, and reported findings to the Study Director and to Management.

Phase of study/ inspection	Date of inspection	Report to Study Director and to Management
Protocol:	Jan. 17, 1994	Jan. 18, 1994
Conduct of study:	Jan. 20, 1994	Jan. 21, 1994
Audit of the report:	Nov. 08, 1994	Nov. 08, 1994

Remarks: Analytics were inspected independently by the Quality Assurance Unit of the analytical laboratory.

Ludwigshafen, Nov. 14, 1994

.....
Dr.rer.nat. H. Fleig
(Head of Quality Assurance Unit)

Project No.: 32M0246/934164

CONTENTS

2. INTRODUCTION

The aim of the present study was to assess the potential of the test substance Uvinul T 150 or its metabolite(s) to induce structural and/or numerical chromosomal aberrations. For this purpose, an in vitro cytogenetic assay was carried out for measuring chromosome aberration frequencies in V79 cells (1, 2). The study was carried out in January 1994, (1st experiment) and in March 1994 (2nd experiment) in accordance with the following guidelines:

- OECD Guideline for Testing of Chemicals - "Genetic Toxicology: In Vitro Mammalian Cytogenetic Test", No. 473.

- EEC Directive 92/69, B 10, Mutagenicity (In Vitro Mammalian Cytogenetic Test).

3. MATERIAL AND METHODS

3.1. TEST SUBSTANCE

Name of test substance:	Uvinul T 150
Batch No.:	08-0083
Test substance No.:	93/246
Appearance, consistency:	White powder
Degree of purity/ Composition:	See test substance characterization dated December 14, 1993
Date of manufacturing:	August 3, 1993
Storage:	Refrigerator (protected from light)

More detailed information about the test substance can be found in the raw data and may be requested from the sponsor (BASF Aktiengesellschaft).

3.2. TEST SUBSTANCE ANALYSIS

The stability of the test substance throughout the study period has been proven by reanalysis.

The homogeneity of the test substance was guaranteed by mixing before preparation of the test substance formulations.

Project No.: 32M0246/934164

1. SUMMARY

The substance Uvinul T 150 was assessed for its potential to induce structural and/or numerical chromosomal aberrations in V79 cells in vitro both in the presence and absence of a metabolizing system.

According to pretests for the determination of the experimental doses the test substance did not exhibit any pronounced toxicity up to 100 µg/ml culture medium, which is above the solubility limit. Thus, 100 µg/ml was selected as top dose both in the experiment with and without metabolic activation (18 hours and 28 hours sampling times), 33 µg/ml and 10 µg/ml culture medium (18 hours sampling time only) were evaluated as further doses.

Chromosomes were prepared 18 hours (low, intermediate and top dose) and 28 hours (top dose only) after test substance treatment, which lasted for about 4 hours in the experiment with S-9 mix or for about 18 hours without metabolic activation. Duplicate cultures were used for all experimental groups.

About 2 - 3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases of each culture in the case of the test substance and vehicle controls, or 50 cells of each culture in the case of the concurrent positive controls, were analyzed for chromosomal aberrations.

The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line.

Both of the positive control chemicals, i.e. EMS and cyclophosphamide led to the expected increase in the number of cells containing structural chromosomal aberrations.

According to the results of the present study, the test substance did not cause any increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S-9 mix or after adding a metabolizing system. An increase in the frequency of cells containing numerical aberrations was not demonstrated either.

Thus, under the experimental conditions chosen here Uvinul T 150 is considered to has neither a chromosome-damaging (clastogenic) effect nor an aneugenic activity in V79 cells in vitro.

Nov 9, 1994
Dr.rer.nat. H.D. Hoffmann
(Head of Section)

Engelhardt, Nov. 8, 1994
Dr.rer.nat. G. Engelhardt
(Study director)

The stability of the test substance in the vehicle DMSO and in aqua dest. was determined analytically.

The analytical investigations were carried out in the Central Analytical Laboratory of BASF Aktiengesellschaft.

3.3. CELL SYSTEM

3.3.1. Cell line, storage

The V79 cell line (1, 2) being derived from the Chinese hamster has a

- high proliferation rate (doubling time of about 12 - 16 hours)
- high plating efficiency (> 90%)
- stable karyotype (modal number of 22 chromosomes).

Stocks of the V79 cell line (1 ml portions) were maintained at -196°C in liquid nitrogen using 7% DMSO in culture medium as a cryoprotectant. Each batch used for cytogenetic experiments was checked for

- mycoplasma contamination
- karyotype stability
- plating efficiency (incl. vital staining).

3.3.2. Cell culture

Stock solutions were thawn at 37°C in a water bath and volumes of 0.5 ml were transferred into 25 cm^2 plastic flasks which contain about 5.0 ml MEM (Minimal Essential Medium incl. glutamin), supplemented with 10% FCS (Fetal Calf Serum) and antibiotics. Cells were grown at 37°C with 5% CO_2 and > 90% humidity and subcultured twice weekly. Cell monolayers were suspended in culture medium after dispersion with 2.5% trypsin solution (about 0.1 ml).

3.4. TISSUE PREPARATION

3.4.1. S-9 fraction

The S-9 fraction was prepared according to AMES et al. (3).

5 male Sprague-Dawley rats (200 - 300 g) received a single intraperitoneal injection of 500 mg Aroclor 1254 (as a 200 mg/ml solution in peanut oil) per kg body weight and were kept for 5 days.

During this time the animals were housed in Makrolon cages and were accommodated in fully air-conditioned rooms in which central air-conditioning guaranteed a range of temperature of 20 - 24°C and a relative humidity of 30 -70%. The day/night rhythm was 12 hours (12 hours light from 6.00 - 18.00 hours and 12 hours darkness from 18.00 - 6.00 hours).

Standardized pelleted feed and tap water from bottles were available ad libitum.

5 days after administration the rats were sacrificed and the livers were prepared (all preparation steps for obtaining the liver microsome enzymes were carried out using sterile solvents and glassware at a temperature of +4°C). The livers were weighed and washed in a weight equivalent volume of a 150 mM KCl solution (1 ml ≙ 1 g wet liver), then cut into small pieces and homogenized in three volumes of KCl solution. After centrifugation of the homogenate at 9000 x g for 10 minutes at +4°C, 5-ml portions of the supernatant (so-called S-9 fraction) were quickly deep-frozen in dry ice and stored at -70°C to -80°C.

3.4.2. S-9 mix

The S-9 mix was prepared freshly prior to each experiment (3). For this purpose, a sufficient amount of S-9 fraction was thawed at room temperature, and 1 volume of S-9 fraction was mixed with 9 volumes of S-9 supplement (cofactors). This preparation, the so-called S-9 mix, was kept on ice until used. The concentrations of the cofactors in the S-9 mix were:

MgCl2	8 mM
KCl	33 mM
glucose-6-phosphate	5 mM
NADP	4 mM
phosphate buffer (pH 7.4)	15 mM.

The phosphate buffer (4) was prepared by mixing an Na_2HPO_4 solution with an NaH_2PO_4 solution at a ratio of about 4 : 1.

3.5. EXPERIMENTAL PERFORMANCE

3.5.1. Pretest for dose selection

- **1st Experiment**

The doses for the 1st experiment (18 hours sampling time) were determined from appropriate pretests with cultures exposed for the duration of 4 hours to a wide dose range of the test article, i.e. 0.1 µg/ml - 100 µg/ml culture medium both without S-9 mix and with S-9 mix. In the course of this, various parameters were checked for all or at least for some selected doses; the results were given in the tables on pages 10 and 11. As a rule for non-toxic test substances the highest dose concentration should not exceed a limit of 5 mg/ml as recommended by the EEC Directive 92/69, B10. or 10 mM as recommended by the OECD and by an ICPEMC Task group (5).

Up to a dose of 100 µg/ml, at which an evident substance precipitation was observed the test substance exhibited only a marginal toxic effect (slight decrease in the number of cells) after a treatment time of 4 hours. Thus, for the experimental part without metabolic activation a further pretest (1 µg/ml - 100 µg/ml) was carried out with a treatment time of 18 hours (see table on page 12).

According to the findings of the pretests, 100 µg/ml without S-9 mix and with metabolic activation were selected as top doses. This selection was based on the solubility of the test substance.

Project No.: 32M0246/934164

18 hours harvest time; treatment for 4 hours without S-9 mix

Dose groups	pH	Osmolality	Solubility		Plating efficiency	Cell counts	Mitotic index	Quality of metaphases
		mOsm	veh	cul	%	%	%	
vehicle control 50 µl DMSO	7.6	449	-	-	-	100	100	*
0.1 µg/ml	-	-	s	s	-	104	-	*
0.5 µg/ml	-	-	s	s	-	99	-	*
1.0 µg/ml	-	-	s	s	-	111	-	*
5.0 µg/ml	-	-	s	s	-	106	-	*
10.0 µg/ml	-	-	s	s	-	64	-	*
50.0 µg/ml	7.7	433	s	p	-	53	92	*
100.0 µg/ml	7.7	465	s	p	-	51	100	*

veh = vehicle
cul = cell culture
s = complete solubility
p = precipitation

* Sufficient metaphases of good quality

Cell attachment to the slides:

Complete attachment of cells to the slides as indicated by cell morphology, i.e. fibroblast - like cells.

Project No.: 32M0246/934164

18 hours harvest time; treatment for 4 hours with S-9 mix

Dose groups	pH	Osmolality m0sm	Solubility veh	Solubility cul	Plating efficiency %	Cell counts %	Mitotic index %	Quality of metaphases
vehicle control 50 µl DMSO	7.3	419	-	-	-	100	100	*
0.1 µg/ml	-	-	s	s	-	91	-	*
0.5 µg/ml	-	-	s	s	-	85	-	*
1.0 µg/ml	-	-	s	s	-	78	-	*
5.0 µg/ml	-	-	s	s	-	73	-	*
10.0 µg/ml	-	-	s	s	-	71	-	*
50.0 µg/ml	7.4	424	s	p	-	74	99	*
100.0 µg/ml	7.4	427	s	p	-	73	86	*

veh = vehicle
cul = cell culture
s = complete solubility
p = precipitation

* Sufficient metaphases of good quality

Cell attachment to the slides:

Complete attachment of cells to the slides as indicated by cell morphology, i.e. fibroblast-like cells.

18 hours harvest time; treatment for 18 hours without S-9 mix

Dose groups	pH	Osmolality mOsm	Solubility veh	Solubility cul	Plating efficiency %	Cell counts %	Mitotic index %	Quality of metaphases
vehicle control 50 µl DMSO	7.6	462	-	-	100	100	100	*
1.0 µg/ml	7.7	456	s	s	-	123	-	*
5.0 µg/ml	7.7	463	s	s	98	111	-	*
10.0 µg/ml	7.6	462	s	s	99	128	-	*
50.0 µg/ml	7.6	467	s	p	96	64	81	*
100.0 µg/ml	7.7	455	s	p	93	98	81	*

veh = vehicle
cul = cell culture
s = complete solubility
p = precipitation

* Sufficient metaphases of good quality

Cell attachment to the slides:

Complete attachment of cells to the slides as indicated by cell morphology, i.e. fibroblast - like cells.

- **1st Experiment**

  Thus, for the 1st experiment the following doses were selected:

Doses without S-9 mix		**Harvest times**
10 µg/ml	(0.012 mM)	18 hours
33 µg/ml	(0.040 mM)	18 hours
100 µg/ml	(0.122 mM)	18 hours

Doses with S-9 mix		**Harvest times**
10 µg/ml	(0.012 mM)	18 hours
33 µg/ml	(0.040 mM)	18 hours
100 µg/ml	(0.122 mM)	18 hours

In general, three dose levels were assessed.

- **2nd Experiment**

  For the 2nd experiment the following doses were selected:

Doses without S-9 mix		**Harvest times**
10 µg/ml	(0.012 mM)	18 hours
33 µg/ml	(0.040 mM)	18 hours
100 µg/ml	(0.122 mM)	18 hours
33 µg/ml*	(0.040 mM)	28 hours
100 µg/ml	(0.122 mM)	28 hours

Doses with S-9 mix		**Harvest times**
10 µg/ml	(0.012 mM)	18 hours
33 µg/ml	(0.040 mM)	18 hours
100 µg/ml	(0.122 mM)	18 hours
33 µg/ml*	(0.040 mM)	28 hours
100 µg/ml	(0.122 mM)	28 hours

This selection was based on the findings of the 1st cytogenetic experiment. Again, three dose levels were assessed at a sampling time of 18 hours. At the additional later harvest time of 28 hours only one dose was evaluated both with and without metabolic activation; the additionally selected lower doses (marked with *) in the experiments both with and without S-9 mix, i.e. 33 µg/ml were planned to be evaluated only if due to cytotoxicity a metaphase analyses is not possible at the selected top doses.

3.5.2. Cell cycle time

The cell cycle of the untreated V79 cells lasted for about 13 - 14 hours (last measurement: December 1993) under the selected cultur conditions. Thus, the selected 1st sampling time of 18 hours (see item 3.5.3.) is within the one to 1.5 x of the normal cell cycle time, as recommended in an "EG Guidance Note - The practical interpretation of Ames V Test Method B 10, the in vitro mammalian cell cytogenetic test." The later sampling time of 28 hours was chosen to cover for possible cell cycle delay.

3.5.3. Sampling times

Chromosomal aberrations were generally analyzed in the first metaphase after they were formed to avoid loss during mitoses or conversion of the initial aberrations into more complex derivatives during subsequent cell cycles.

Since aberrations were induced by the majority of chemical clastogens during DNA replication, harvest time must allow cells to progress through S-phase after treatment to convert initial DNA damage into chromosome alterations visible at mitosis.

Because V79 cells are asynchronous and different chemicals might affect different stages of the cell cycle more than one sampling time is necessary. Furthermore, mitotic delay may result from clastogen exposure and thus considerably delay the first post-treatment mitosis.

Thus, samples were taken at 18 hours (low, intermediate and top dose) and 28 hours (top dose only) after the beginning of a 4-hour treatment (with S-9 mix) or of a 18-hour treatment (without S-9 mix) covering the intervals in which maximum aberration frequencies were expected.

3.5.4. Test groups and doses - 1st experiment

The number of test groups selected according to the pretest and evaluated in the 1st main cytogenetic experiment can be seen from the following table. Duplicate cultures were used for all experimental groups.

Test group No. 18 h	S-9 mix	Doses µl/ml and/or µg per ml culture medium	Metaphases analyzed 18 h
1	-	vehicle control 50 µl DMSO	200
2	-	10 µg	200
3	-	33 µg	200
4	-	100 µg	200
5	-	350 µg EMS	100
6	+	vehicle control 50 µl DMSO	200
7	+	10 µg	200
8	+	33 µg	200
9	+	100 µg	200
10	+	0.5 µg cyclophosphamide	100

3.5.5. Test groups and doses - 2nd experiment

The number of the dose groups selected according to the findings of the 1st experimental and evaluated in the 2nd cytogenetic experiment can be seen from the following table. Again, duplicate cultures were used for all experimental groups.

Test group No. 18 h	Test group No. 28 h	S-9 mix	Doses µl/ml and/or µg per ml culture medium	Metaphases analyzed 18 h	Metaphases analyzed 28 h
11		-	vehicle control 50 µl DMSO	200	
12		-	10 µg	200	
13		-	33 µg	200	
14		-	100 µg	200	
15			350 µg EMS	100	
16		+	vehicle control 50 µl DMSO	200	
17		+	10 µg	200	
18		+	33 µg	200	
19		+	100 µg	200	
20		+	0.5 µg cyclophosphamide	100	
			vehicle control		
	21	-	50 µl DMSO		200
	22	-	100 µg		200
			vehicle control		
	23	+	50 µl DMSO		200
	24	+	100 µg		200

3.5.6. Control articles

Vehicle controls

The vehicle controls with and without S-9 mix only contained the vehicle for the test substance at the same concentration and volume used in the test culture.

Positive controls

The following positive control substances were used to demonstrate the sensitivity of the test method and the activity of the S-9 mix:

- Without metabolic activation 350 µg ethyl-methane-sulfonate (EMS)/ml culture medium added in a volume of 1.0 ml

- With metabolic activation (S-9 mix) 0.5 µg cyclophosphamide (CPP)/ml culture medium added in a volume of 1.0 ml

3.5.7. Preparation of test cultures

- Logarithmically growing cultures more than 50% confluent were trypsinized (2.5% trypsin solution and Ca-Mg-free Hanks Balanced Salt Solution HBSS). Prior to trypsin treatment the cells were rinsed once with 5 ml Ca-Mg-free HBSS.
- This process was stopped by adding MEM supplemented with 10% FCS.
- A single suspension was prepared and about 5 ml MEM supplemented with 10% FCS and containing about 30 000 - 50 000 cells were seeded in each chamber of Quadriperm dishes. Two chambers of a Quadriperm dish were used for one test culture.
- The Quadriperm dishes were incubated at 37°C with 5% CO_2 and > 90% humidity.

3.5.8. Treatment of the test cultures

24 hours after seeding and incubating the cells the medium was replaced by fresh medium. The test article, dissolved in 50 µl DMSO, was added to the culture medium with or without 1 ml S-9 mix. Concurrent negative and positive controls (see item 3.5.6.) were tested in parallel.

After incubation (37°C, 5% CO_2, > 90% humidity) for 4 hours with S-9 mix the serum-free medium was replaced by MEM supplemented with 10% FCS after rinsing twice with Hanks balanced salt solution (HBSS). Subsequently, the Quadriperm dishes were incubated again for another 14 hours or 24 hours until the cells were harvested. Without S-9 mix cells were treated for 18 hours in culture medium supplemented with 10% FCS.

3.5.9. Cell harvest and preparation of metaphase spreads

The cells were prepared based on the methods described by SCHMID, W. (6) and SPEIT, G. and S. HAUPTER (7).

- 2 - 3 hours prior to harvesting the cells, 0.2 µg colcemid/ml culture medium (= 1 µg Colcemid dissolved in 0.1 ml PBS/culture) was added in each chamber in order to arrest mitosis in the metaphase.
- After incubation at 37°C the culture medium was completely removed.
- For hypotonic treatment 5 ml of a 0.4% KCl solution which was at 37°C was added for about 20 minutes.
- Subsequently 5 ml of fixative (methanol : glacial acetic acid/3 : 1) which was at 4°C was added and kept for at least 15 minutes and then replaced. After about another 10 minutes fixative was replaced again and kept for at least 5 minutes at room temperature for complete fixation.
- The slides were taken out of the Quadriperm chambers, briefly dripped off and than rapidly passed through a Bunsen burner flame.
- The preparations were dried in the air and subsequently stained in a solution of Giemsa and Titrisol (15 ml Giemsa, 185 ml Titrisol pH 7.2) for 10 minutes.
- After being rinsed twice in aqua dest. and clarified in xylene, the preparations were mounted in Corbit-Balsam.

3.6. EVALUATION

3.6.1. Chromosome analysis

As a rule, the first 100 consecutive well-spread metaphases of each culture were counted for all test groups and if cells had 20 - 22 chromosomes, they were analyzed for chromosome aberrations according to the following definitions (8, 9, 10):

- **Structural chromosome aberrations**

- G' and G"	chromatid gap and isochromatid gap unstained regions (so-called achromatic lesions) without dislocation of the segment which appears to be separated.
- B' and B"	chromatid break and chromosome break visible discontinuity in chromatid or chromosome structure with lateral or longitudinal dislocation of the fragment.
- F' and F"	chromatid fragment and chromosome fragment acentric chromosome segments which occur singly or in pairs.
- D' and D"	chromatid deletion and chromosome deletion loss of a segment on the level of chromatids or chromosomes.
- m. A.	multiple aberrations metaphases with 5 or more aberrations excl. gaps.
- disintegration of chromosomal (pulverization = P):	the chromosomes being present as irregular particles, a chromosomal structure cannot be detected any longer.

- Exchanges (translocations)

  These exchange aberrations (Ex) are divided into intrachanges and interchanges:

  - Int' and Int" — intrachanges on the level of chromatids and chromosomes

    the joining of broken ends capable of reuniting two or several chromatid regions within a chromosome, e.g., centric ring chromosomes, pericentric inversions.

  - I' and I" — interchanges on the level of chromatids and chromosomes

    the joining of broken ends capable of reuniting two or several chromosomes. They are classified as:

    - symmetric interchanges, e.g., reciprocal translocations between nonhomologous chromosomes, centric fusions, quadriradial structures
    - asymmetric interchanges, e.g., dicentric and polycentric chromosomes, triradial and quadriradial structures.

- **Numerical chromosome aberrations (so-called heteroploidies)**

  - Aneuploidy — metaphases with absent (hypoploid) or additional (hyperploid) chromosomes

    Only hyperploid metaphases are registered.

  - Euploidy (= polyploidy) — changes in the number of chromosomes by whole chromosome sets.

Slides were coded before microscopic analysis. If only a few cells were found or if the metaphases were of low quality, a chromosome analysis was not carried out.

In cases of a clear increase in chromosomally damaged cells the number of metaphases to be analyzed are reduced from the intended 200 mitoses/test group.

3.6.2. Mitotic index

A mitotic index based on 1500 cells/culture was determined for all test in both experiments.

3.6.3. Cell counts

For determination of cytotoxicity additional cell cultures (using 25 cm^2 plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by counting the number of cells in the dose groups in comparison to the concurrent vehicle control at the end of the culture period using a counting chamber.

3.6.4. Cell morphology

About 3 hours after test substance treatment cultures of all test groups were checked regarding cell morphology, which is an indication of attachment of the cells to the slides.

3.6.5. Treatment conditions

pH values and osmolality were measured. The solubility of the test substance in the vehicle used and in the aqueous culture medium was checked to ensure proper culturing and to avoid extreme treatment conditions (5).

3.7. STATISTICAL EVALUATION

The statistical evaluation of the data was carried out using the MUCHAN program system (BASF AG).

For each group the proportion of metaphases with aberrations was calculated.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected over the dose groups separately for each time point and was performed one-sided. If the results of this test are significant, labels (* $p < 0.05$, ** $p < 0.01$) were printed in the tables.

3.8. RETENTION OF RECORDS

The raw data, protocol, reserve sample and microscopic preparations as well as the original of this report will be stored at BASF Aktiengesellschaft at least for the period of time specified in the GLP regulations. Details concerning responsibilities or locations of archiving can be seen from the respective SOP's and from the raw data.

4. RESULTS

4.1. CHROMOSOME ANALYSIS - 1st EXPERIMENT

(Tables 1 to 12; Appendix 1)

Table 1:	Summary table: results of all groups without S-9 mix; 18 hours harvest time
Table 2:	Summary table: results of all groups with S-9 mix; 18 hours harvest time
Tables 3 - 7:	Results of the individual cultures of each test group without S-9 mix; 18 hours harvest time
Tables 8 - 12:	Results of the individual cultures of each test group with S-9 mix; 18 hours harvest time

4.1.1. Assay without S-9 mix; 18 hours harvest time

Vehicle control:

5 (2.5%) metaphases incl. gaps
No (0.0%) metaphases excl. gaps

No (0.0%) hyperploid cells
No (0.0%) polyploid cells

10 µg/ml:

6 (3.0%) metaphases incl. gaps
2 (1.0%) metaphases excl. gaps, i.e. 1 x F"; 1 x 3F"

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

33 µg/ml:

5 (2.5%) metaphases incl. gaps
2 (1.0%) metaphases excl. gaps, i.e. 1 x B'; 1 x Ex

1 (0.5%) hyperploid cell
1 (0.5%) polyploid cell

100 µg/ml:

3 (1.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x Ex

1 (0.5%) hyperploid cell
No (0.0%) polyploid cells

350 µg EMS/ml:

With 17.0 % aberrant cells incl. gaps and 14.0% aberrant mitosis excl. gaps including 10.0% cells with exchanges, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

4.1.2. Assay with S-9 mix; 18 hours harvest time

Vehicle control:

5 (2.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x Ex

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

10 µg/ml:

5 (2.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x D"

No (0.0%) hyperploid cells
3 (1.5%) polyploid cells

33 µg/ml:

7 (3.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x D"

No (0.0%) hyperploid cells
2 (1.0%) polyploid cells

100 µg/ml:

3 (1.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x Ex

1 (0.5%) hyperploid cell
No (0.0%) polyploid cells

0.5 µg cyclophosphamide/ml:

With 18.0 % aberrant cells incl. gaps and 15.0% aberrant metaphases excl. gaps including 8.0% cells with exchanges, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

4.2. CHROMOSOME ANALYSIS - 2nd EXPERIMENT

(Tables 13 to 30; Appendix 2)

Table 13:	Summary table: results of all groups without S-9 mix; 18 hours harvest time
Table 14:	Summary table: results of all groups without S-9 mix; 28 hours harvest time
Table 15:	Summary table: results of all groups with S-9 mix; 18 hours harvest time
Table 16:	Summary table: results of all groups with S-9 mix; 28 hours harvest time
Tables 17 - 21:	Results of the individual cultures of each test group without S-9 mix; 18 hours harvest time
Tables 22 - 23:	Results of the individual cultures of each test group without S-9 mix; 28 hours harvest time
Tables 24 - 28:	Results of the individual cultures of each test group with S-9 mix; 18 hours harvest time
Tables 29 - 30:	Results of the individual cultures of each test group with S-9 mix; 28 hours harvest time

4.2.1. **Assay without S-9 mix; 18 hours harvest time**

Vehicle control:

5 (2.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x B"

No (0.0%) hyperploid cells
No (0.0%) polyploid cells

10 µg/ml:

3 (1.5%) metaphases incl. gaps
3 (1.5%) metaphases excl. gaps, i.e. 1 x B'; 1 x B"; 1 x D"

1 (0.5%) hyperploid cell
2 (1.0%) polyploid cells

33 µg/ml:

1 (0.5%) metaphase incl. gaps
No (0.0%) metaphases excl. gaps

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

100 µg/ml:

6 (3.0%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x Ex

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

350 µg EMS/ml:

With 11.0% aberrant cells incl. gaps and 11.0% aberrant mitosis excl. gaps including 7.0% cells with exchanges, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

4.2.2. **Assay without S-9 mix; 28 hours harvest time**

Vehicle control:

5 (2.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x F"

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

100 µg/ml:

7 (3.5%) metaphases incl. gaps
3 (1.5%) metaphases excl. gaps, i.e. 1 x B"; 2 x Ex

No (0.0%) hyperploid cells
No (0.0%) polyploid cells

4.2.3. **Assay with S-9 mix; 18 hours harvest time**

Vehicle control:

6 (3.0%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x B'

No (0.0%) hyperploid cells
No (0.0%) polyploid cells

10 µg/ml:

8 (4.0%) metaphases incl. gaps
3 (1.5%) metaphases excl. gaps, i.e. 1 x F"; 2 x Ex

No (0.0%) hyperploid cells
3 (1.5%) polyploid cells

33 µg/ml:

5 (2.5%) metaphases incl. gaps
1 (0.5%) metaphase excl. gaps, i.e. 1 x B"

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

100 µg/ml:

4 (2.0%) metaphases incl. gaps
3 (1.5%) metaphases excl. gaps, i.e. 2 x B'; 1 x m.A. incl. Ex

No (0.0%) hyperploid cells
1 (0.5%) polyploid cell

0.5 µg cyclophosphamide/ml:

With 14.0% aberrant cells incl. gaps and 14.0% aberrant metaphases excl. gaps including 6.0% cells with exchanges, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

4.2.4. Assay with S-9 mix; 28 hours harvest time

Vehicle control:

6 (3.0%) metaphases incl. gaps
3 (1.5%) metaphases excl. gaps, i.e. 1 x B"; 2 x Ex

No (0.0%) hyperploid cells
No (0.0%) polyploid cells

100 µg/ml:

13 (6.5%) metaphases incl. gaps
7 (3.5%) metaphases excl. gaps, i.e. 2 x B'; 3 x D"; 1 x Ex; 1 x P

No (0.0%) hyperploid cells
No (0.0%) polyploid cells

4.3. MITOTIC INDEX

The mitotic index based on 1500 cells per culture for the different test groups without and with metabolic activation can be seen on the following tables. The numbers of mitotic cells in the samples scored are given as "absolute" values. The "relative" figures are related to the corresponding vehicle controls which are set 100%.

According to this, no suppression of the mitotic activity was observed under any of the experimental conditions.

- **1st Experiment**

Test groups 18 hours	S-9 mix	1st culture % abs.	2nd culture % abs.	Mean % abs.	% rel.
vehicle control 50 µl DMSO	-	17.2	10.8	14.0	100
10 µg/ml	-	8.4	11.0	9.7	69.3
33 µg/ml	-	17.6	8.8	13.2	94.3
100 µg/ml	-	8.4	5.0	6.7	47.9
vehicle control 50 µl DMSO	+	18.5	18.7	18.6	100
10 µg/ml	+	15.3	15.7	15.5	83.3
33 µg/ml	+	14.1	15.9	15.0	80.7
100 µg/ml	+	19.3	11.9	15.6	83.9

•**2nd Experiment**

Test groups 18 hours	S-9 mix	1st culture % abs.	2nd culture % abs.	Mean % abs.	% rel.
vehicle control 50 µl DMSO	-	9.1	8.7	8.9	100
10 µg/ml	-	10.5	14.5	12.5	140.4
33 µg/ml	-	12.8	13.3	13.1	147.2
100 µg/ml	-	7.9	7.8	7.9	88.8
vehicle control 50 µl DMSO	+	12.9	11.7	12.3	100
10 µg/ml	+	19.3	14.3	16.8	136.6
33 µg/ml	+	4.8	15.5	10.2	82.9
100 µg/ml	+	14.2	14.7	14.5	117.9

Test groups 28 hours	S-9 mix	1st culture % abs.	2nd culture % abs.	Mean % abs.	% rel.
vehicle control 50 µl DMSO	-	9.1	6.8	8.0	100
100 µg/ml	-	11.5	8.2	9.9	123.8
vehicle control 50 µl DMSO	+	6.7	6.9	6.8	100
100 µg/ml	+	13.7	14.9	14.3	210.3

4.4. CELL COUNTS

The results of the cytotoxicity test cell count can be seen from the following tables. The number of cells counted in the dose groups are expressed as a percentage of the concurrent vehicle control value. According to this, no growth inhibition was observed under any of the experimental conditions.

- **1st Experiment**

Test groups	S-9 mix	Harvest time 18 hours No. of cells 5×10^3/ml	%
vehicle control 50 µl DMSO	-	74	100
10 µg/ml	-	80	108.1
33 µg/ml	-	62	83.8
100 µg/ml	-	86	116.2
vehicle control 50 µl DMSO	+	84	100
10 µg/ml	+	76	90.5
33 µg/ml	+	83	98.8
100 µg/ml	+	85	101,2

- **2nd Experiment**

Test groups	S-9 mix	Harvest time			
		18 hours		28 hours	
		No. of cells 5×10^3/ml	%	No. of cells 5×10^3/ml	%
vehicle control 50 µl DMSO	-	90	100	104	100
10 µg/ml	-	94	104.4		
33 µg/ml	-	84	93.3		
100 µg/ml	-	88	97.8	100	96.2
vehicle control 50 µl DMSO	+	104	100	124	100
10 µg/ml	+	79	76.0		
33 µg/ml	+	81	77.9		
100 µg/ml	+	78	75.0	102	82.3

4.5. CELL MORPHOLOGY

- **1st Experiment**

Test groups	S-9 mix	Cell morphology	Attachment to slides
vehicle control 50 µl DMSO	-	fibroblast - like cells	complete attachment
10 µg/ml	-	fibroblast - like cells	complete attachment
33 µg/ml	-	fibroblast - like cells	complete attachment
100 µg/ml	-	fibroblast - like cells	complete attachment
vehicle control 50 µl DMSO	+	fibroblast - like cells	complete attachment
10 µg/ml	+	fibroblast - like cells	complete attachment
33 µg/ml	+	fibroblast - like cells	complete attachment
100 µg/ml	+	fibroblast - like cells	complete attachment

- **2nd Experiment**

Test groups	S-9 mix	Cell morphology	Attachment to slides
vehicle control 50 µl DMSO	-	fibroblast - like cells	complete attachment
10 µg/ml	-	fibroblast - like cells	complete attachment
33 µg/ml	-	fibroblast - like cells	complete attachment
100 µg/ml	-	fibroblast - like cells	complete attachment
vehicle control 50 µl DMSO	+	fibroblast - like cells	complete attachment
10 µg/ml	+	fibroblast - like cells	complete attachment
33 µg/ml	+	fibroblast - like cells	complete attachment
100 µg/ml	+	fibroblast - like cells	complete attachment

4.6. TREATMENT CONDITIONS

The osmolality and pH values and the observations regarding the solubility of the test substance can be seen from the following tables.

- **1st Experiment**

Test groups 18 hours	S-9 mix	pH	Osmolality m0sm	Solubility vehicle	Solubility culture medium
vehicle control 50 µl DMSO	-	8.0	394	-	-
10 µg/ml	-	8.0	425	s	s
33 µg/ml	-	8.1	415	s	p
100 µg/ml	-	8.0	434	s	p
vehicle control 50 µl DMSO	+	8.0	424	-	-
10 µg/ml	+	8.0	431	s	s
33 µg/ml	+	8.0	433	s	p
100 µg/ml	+	8.1	432	s	p

s = compete solubility
p = precipitation

- **2nd Experiment**

Test groups 18 hours	S-9 mix	pH	Osmolality mOsm	Solubility vehicle	 culture medium
vehicle control 50 µl DMSO	-	8.2	516	-	-
10 µg/ml	-	8.3	437	s	s
33 µg/ml	-	8.2	457	s	p
100 µg/ml	-	8.3	453	s	p
vehicle control 50 µl DMSO	+	7.8	438	-	-
10 µg/ml	+	7.8	424	s	s
33 µg/ml	+	8.0	403	s	p
100 µg/ml	+	7.8	413	s	p

Test groups 28 hours	S-9 mix	pH	Osmolality mOsm	Solubility vehicle	 culture medium
vehicle control 50 µl DMSO	-	7.8	440	-	-
100 µg/ml	-	7.8	441	s	p
vehicle control 50 µl DMSO	+	7.6	373	-	-
100 µg/ml	+	7.6	407	s	p

s = complete solubility
p = precipitation

4.7. TEST SUBSTANCE ANALYSIS

The stability of the test substance in the vehicle over a period of 4 hours and in aqua dest. over a period of 96 hours was verified analytically.

With the vehicle DMSO a solution was obtained and therefore, it was not necessary to verify the homogeneity analytically.

5. DISCUSSION AND CONCLUSIONS

According to the results of the present in vitro cytogenetic study, the test substance Uvinul T 150 did not lead to an increase in the number of structural chromosomal aberrations incl. and excl. gaps either without S-9 mix or after the addition of a metabolizing system; types and frequency of aberrations were nearly the range of that of the concurrent negative control values at both sampling times and within the range of the historical control data i.e. 1.5% - 6.0% (without S-9 mix) or 4.5% - 10.5% (with S-9 mix) incl. gaps and 0.5% - 3.5% (without S-9 mix) or 1.0% - 5.0% (with S-9 mix) excl. gaps.

An increase in the number of cells containing numerical chromosomal aberrations was not demonstrated either.

Thus, under the experimental conditions chosen here Uvinul T 150 is considered neither to be a chromosome-damaging (clastogenic) agent nor to have any aneugenic activity under in vitro conditions using V79 cells.

6. LITERATURE

1. BRADLEY, M.O.; BHUYAN, B.; FRANCIS, M.C.; LANGENBACH, R.; PETERSON, A.; HUBERMAN, E.:

Mutagenesis by chemical agents in V79 Chinese hamster cells: A review and analysis in literature. A report of the Gene-Tox Program.

Mut. Res., 87, 81 - 142 (1981)

2. PRESTON, R.J.; AU, W.; BENDER, M.A.; BREWEN, J.G.; CARRANO, A.V.; HEDDLE, J.A.; McFEE, R.F.; WOLFF, S.; WASSOM, J.S.:

Mammalian in vivo and in vitro cytogenetic assays: A report of the U.S. EPA's Gene-Tox Program.

Mut. Res., 87, 143 - 188 (1981)

3. AMES, B.N.; McCANN, J.; YAMASAKI, E.:

Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test.

Mut. Res., 31, 347 - 364 (1975)

4. DeMARINI; D.M., DALLAS, M.M., LEWTAS, J.:

Cytotoxicity and effect on mutagenicity of buffers in a microsuspension assay.

Ter. Canc. Mut., 9, 287 - 295 (1989)

5. SCOTT, D.; GALLOWAY, S.M.; MARSHALL, R.R.; ISHIDATE Jr., M.; BRUSICK, D.; ASHBY, J.; MYHR, B.C.:

Genotoxicity under extreme culture conditions. A report from ICPEMC Task Group 9.

Mut. Res., 257, 147 - 204 (1991)

6. SCHMID, W.:

A technique for in situ karyotyping of primary amniotic fluid cell cultures.

Humangenetik, 30, 325 - 330 (1975)

7. SPEIT, G.; HAUPTER, S.:

Cytogenetic effects of penicillamine.

Mut. Res., 190, 197 - 203 (1987)

8. EVANS, H.J.; O'RIORDAN, M.L.:

Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests.

Mut. Res., 31, 135 - 148 (1975)

9. SAVAGE, J.R.K.:

Classification and relationships of induced chromosomal structural changes.

J. Med. Genet., 12, 103 - 122 (1975)

10. Standard-Protokoll zur cytogenetischen Auswertung von Mitose- und Meiosechromosomen bei der Routineuntersuchung; ausgearbeitet von der "Arbeitsgruppe der Industrie, 'Cytogenetik'", 1987

11. SPEIT, G.; HAUPTER, S.; VOGEL, W.:

Characterization of mitosis with sister chromatid differentiation (SCD) and consequences for the analysis of proliferation kinetics and sister chromatid exchanges in asynchronously growing cells.

Hum. Genet., 71, 358 - 360 (1985)

Project No.: 32M0246/934164

Appendix 1

1st EXPERIMENT

(Tables 1 - 12)

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
SUMMARY TABLE (WITHOUT S9-MIX) 27-JUN-94

DOSE		H. CULTURES	METAPHASES	METAPHASES WITH ABERRATIONS											
				INCL.GAPS		EXCL.GAPS		EXCHANGES		MUL.ABER.		CHR. DIS.		ANEUPL.		POLYPL.	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
VEHICLE DMSO	:	18 2	200	5	2.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
10 UG/ML	:	18 2	200	6	3.0	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5
33 UG/ML	:	18 2	200	5	2.5	2	1.0	1	0.5	0	0.0	0	0.0	1	0.5	1	0.5
100 UG/ML	:	18 2	200	3	1.5	1	0.5	1	0.5	0	0.0	0	0.0	1	0.5	0	0.0
EMS 350 UG/ML	:	18 2	100	17	17.0**	14	14.0**	10	10.0**	0	0.0	0	0.0	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP ,BONFERRONI-HOLM CORRECTED FOR EACH TIME

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
SUMMARY TABLE (WITH S9-MIX) 27-JUN-94

DOSE		H. CULTURES	METAPHASES	METAPHASES WITH ABERRATIONS											
				INCL.GAPS		EXCL.GAPS		EXCHANGES		MUL.ABER.		CHR. DIS.		ANEUPL.		POLYPL.	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
VEHICLE DMSO	:	18 2	200	5	2.5	1	0.5	1	0.5	0	0.0	0	0.0	0	0.0	1	0.5
10 UG/ML	:	18 2	200	5	2.5	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	3	1.5
33 UG/ML	:	18 2	200	7	3.5	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	2	1.0
100 UG/ML	:	18 2	200	3	1.5	1	0.5	1	0.5	0	0.0	0	0.0	1	0.5	0	0.0
CPP 0.5 UG/ML	:	18 2	100	18	18.0**	15	15.0**	8	8.0**	0	0.0	0	0.0	0	0.0	1	1.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP ,BONFERRONI-HOLM CORRECTED FOR EACH TIME

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
VEHICLE DMSO																				
	004	18	-	0100	0258	17.2	3	3.0	0		0		0		0		0		0	
	008	18	-	0100	0162	10.8	2	2.0	0		0		0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL. GAPS N	INCL. GAPS %	EXCL. GAPS N	EXCL. GAPS %	EXCHANGES N	EXCHANGES %	MUL. ABER. N	MUL. ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
10 UG/ML																				
	009	18	-	0100	0126	8.4	4	4.0	1	1.0	0		0		0		0		0	
	014	18	-	0100	0165	11.0	2	2.0	1	1.0	0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
33 UG/ML																				
	012	18	-	0100	0264	17.6	1	1.0	0		0		0		0		1	1.0	0	
	020	18	-	0100	0132	8.8	4	4.0	2	2.0	1	1.0	0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
100 UG/ML																				
	001	18	-	0100	0126	8.4	3	3.0	1	1.0	1	1.0	0		0		0		0	
	017	18	-	0100	0075	5.0	0		0		0		0		0		1	1.0	0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
EMS 350 UG/ML																				
	002	18	-	0050	0000		8	16.0	7	14.0	4	8.0	0		0		0		0	
	016	18	-	0050	0000		9	18.0	7	14.0	6	12.0	0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
VEHICLE DMSO																				
	003	18	+	0100	0277	18.5	3	3.0	1	1.0	1	1.0	0		0		0		0	
	005	18	+	0100	0281	18.7	2	2.0	0		0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT.INDEX N	%	INCL.GAPS N	%	EXCL.GAPS N	%	EXCHANGES N	%	MUL.ABER. N	%	CHR. DIS. N	%	ANEUPL. N	%	POLYPL. N	%
10 UG/ML																				
	010	18	+	0100	0229	15.3	2	2.0	1	1.0	0		0		0		0		1	1.0
	013	18	+	0100	0235	15.7	3	3.0	0		0		0		0		0		2	2.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
33 UG/ML																				
	006	18	+	0100	0212	14.1	4	4.0	0		0		0		0		0		0	
	018	18	+	0100	0238	15.9	3	3.0	1	1.0	0		0		0		0		2	2.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
100 UG/ML																				
	011	18	+	0100	0289	19.3	1	1.0	1	1.0	1	1.0	0		0		1	1.0	0	
	019	18	+	0100	0178	11.9	2	2.0	0		0		0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

27-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
CPP 0.5 UG/ML																				
	007	18	+	0050	0000		8	16.0	7	14.0	4	8.0	0		0		0		0	
	015	18	+	0050	0000		10	20.0	8	16.0	4	8.0	0		0		0		1	2.0

Project No.: 32M0246/934164

Appendix 2

2nd EXPERIMENT

(Tables 13 - 30)

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
SUMMARY TABLE (WITHOUT S9-MIX)

23-JUN-94

DOSE		H. CULTURES	METAPHASES	METAPHASES WITH ABERRATIONS											
				INCL.GAPS		EXCL.GAPS		EXCHANGES		MUL.ABER.		CHR. DIS.		ANEUPL.		POLYPL.	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
VEHICLE DMSO	:	18 2	200	5	2.5	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
10 UG/ML	:	18 2	200	3	1.5	3	1.5	0	0.0	0	0.0	0	0.0	1	0.5	2	1.0
33 UG/ML	:	18 2	200	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5
100 UG/ML	:	18 2	200	6	3.0	1	0.5	1	0.5	0	0.0	0	0.0	0	0.0	1	0.5
EMS 350 UG/ML	:	18 2	100	11	11.0**	11	11.0**	7	7.0**	0	0.0	0	0.0	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP ,BONFERRONI-HOLM CORRECTED FOR EACH TIME

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
SUMMARY TABLE (WITHOUT S9-MIX) 23-JUN-94

DOSE		H. CULTURES	METAPHASES	METAPHASES WITH ABERRATIONS													
				INCL.GAPS		EXCL.GAPS		EXCHANGES		MUL.ABER.		CHR. DIS.		ANEUPL.		POLYPL.	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
VEHICLE DMSO	:	28 2	200	5	2.5	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5
100 UG/ML	:	28 2	200	7	3.5	3	1.5	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP ,BONFERRONI-HOLM CORRECTED FOR EACH TIME

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
SUMMARY TABLE (WITH S9-MIX) 23-JUN-94

DOSE		H. CULTURES	METAPHASES	METAPHASES WITH ABERRATIONS											
				INCL.GAPS		EXCL.GAPS		EXCHANGES		MUL.ABER.		CHR. DIS.		ANEUPL.		POLYPL.	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
VEHICLE DMSO	:	18 2	200	6	3.0	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
10 UG/ML	:	18 2	200	8	4.0	3	1.5	2	1.0	0	0.0	0	0.0	0	0.0	3	1.5
33 UG/ML	:	18 2	200	5	2.5	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5
100 UG/ML	:	18 2	200	4	2.0	3	1.5	1	0.5	1	0.5	0	0.0	0	0.0	1	0.5
CPP 0.5 UG/ML	:	18 2	100	14	14.0**	14	14.0**	6	6.0**	0	0.0	0	0.0	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP ,BONFERRONI-HOLM CORRECTED FOR EACH TIME

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
SUMMARY TABLE (WITH S9-MIX) 23-JUN-94

DOSE		H. CULTURES	METAPHASES	METAPHASES WITH ABERRATIONS											
				INCL.GAPS		EXCL.GAPS		EXCHANGES		MUL.ABER.		CHR. DIS.		ANEUPL.		POLYPL.	
				N	%	N	%	N	%	N	%	N	%	N	%	N	%
VEHICLE DMSO	:	28 2	200	6	3.0	3	1.5	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0
100 UG/ML	:	28 2	200	13	6.5	7	3.5	1	0.5	0	0.0	1	0.5	0	0.0	0	0.0

FISHER'S EXACT TEST (ONE-SIDED) WITH BONFERRONI-HOLM CORRECTION: *: p <= 0.05, **: p <= 0.01
A PAIRWISE COMPARISON OF EACH DOSE GROUP WITH THE SOLVENT CONTROL GROUP ,BONFERRONI-HOLM CORRECTED FOR EACH TIME

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL. GAPS N	INCL. GAPS %	EXCL. GAPS N	EXCL. GAPS %	EXCHANGES N	EXCHANGES %	MUL. ABER. N	MUL. ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
VEHICLE DMSO																				
	026	18	-	0100	0136	9.1	4	4.0	1	1.0	0		0		0		0		0	
	028	18	-	0100	0130	8.7	1	1.0	0		0		0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
10 UG/ML																				
	053	18	-	0100	0157	10.5	1	1.0	1	1.0	0		0		0		0		1	1.0
	055	18	-	0100	0218	14.5	2	2.0	2	2.0	0		0		0		1	1.0	1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME 59 INT.	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
33 UG/ML																			
	027	18 -	0100	0192	12.8	1	1.0	0		0		0		0		0		0	
	040	18 -	0100	0199	13.3	0		0		0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
100 UG/ML																				
	024	18	-	0100	0118	7.9	4	4.0	1	1.0	1	1.0	0		0		0		0	
	050	18	-	0100	0117	7.8	2	2.0	0		0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT.INDEX N	%	INCL.GAPS N	%	EXCL.GAPS N	%	EXCHANGES N	%	MUL.ABER. N	%	CHR. DIS. N	%	ANEUPL. N	%	POLYPL. N	%
EMS 350 UG/ML																				
	021	18	-	0050	0000		6	12.0	6	12.0	3	6.0	0		0		0		0	
	042	18	-	0050	0000		5	10.0	5	10.0	4	8.0	0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS 23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
VEHICLE DMSO																				
	037	28	-	0100	0136	9.1	4	4.0	1	1.0	0		0		0		0		0	
	051	28	-	0100	0102	6.8	1	1.0	0		0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL. GAPS N	INCL. GAPS %	EXCL. GAPS N	EXCL. GAPS %	EXCHANGES N	EXCHANGES %	MUL. ABER. N	MUL. ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
100 UG/ML																				
	022	28	-	0100	0172	11.5	4	4.0	2	2.0	2	2.0	0		0		0		0	
	030	28	-	0100	0123	8.2	3	3.0	1	1.0	0		0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT.INDEX N	%	INCL.GAPS N	%	EXCL.GAPS N	%	EXCHANGES N	%	MUL.ABER. N	%	CHR. DIS. N	%	ANEUPL. N	%	POLYPL. N	%
VEHICLE DMSO																				
	029	18	+	0100	0194	12.9	4	4.0	0		0		0		0		0		0	
	049	18	+	0100	0176	11.7	2	2.0	1	1.0	0		0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL. GAPS N	INCL. GAPS %	EXCL. GAPS N	EXCL. GAPS %	EXCHANGES N	EXCHANGES %	MUL. N	ABER. %	CHR. N	DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
10 UG/ML																				
	031	18	+	0100	0290	19.3	5	5.0	2	2.0	1	1.0	0		0		0		2	2.0
	033	18	+	0100	0214	14.3	3	3.0	1	1.0	1	1.0	0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
33 UG/ML																				
	047	18	+	0100	0072	4.8	1	1.0	0		0		0		0		0		0	
	048	18	+	0100	0232	15.5	4	4.0	1	1.0	0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
100 UG/ML																				
	034	18	+	0100	0213	14.2	1	1.0	1	1.0	1	1.0	1	1.0	0		0		0	
	054	18	+	0100	0220	14.7	3	3.0	2	2.0	0		0		0		0		1	1.0

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS 23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
CPP 0.5 UG/ML																				
	035	18	+	0050	0000		6	12.0	6	12.0	3	6.0	0		0		0		0	
	039	18	+	0050	0000		8	16.0	8	16.0	3	6.0	0		0		0		0	

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

23-JUN-94

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL.ABER. N	MUL.ABER. %	CHR. DIS. N	CHR. DIS. %	ANEUPL. N	ANEUPL. %	POLYPL. N	POLYPL. %
VEHICLE DMSO																				
	025	28	+	0100	0100	6.7	4	4.0	2	2.0	2	2.0	0		0		0		0	
	052	28	+	0100	0104	6.9	2	2.0	1	1.0	0		0		0		0		0	

BASF/ZHT-TOXICOLOGY

ANALYSIS OF CHROMOSOMES

PROJECT-NO. : 32M0246/934164
SUBSTANCE NAME : UVINUL T 150
CELL TYPE : V79
DETAILED RESULTS

DOSE	CUL. NO.	TIME INT.	S9	META. N	MIT. N	INDEX %	INCL.GAPS N	INCL.GAPS %	EXCL.GAPS N	EXCL.GAPS %	EXCHANGES N	EXCHANGES %	MUL. N	ABER. %	CHR N
100 UG/ML															
	023	28	+	0100	0205	13.7	9	9.0	4	4.0	1	1.0	0		0
	038	28	+	0100	0223	14.9	4	4.0	3	3.0	0		0		1

BASF Abt. Tox.
Abteilung Toxikologie
Department of Toxicology

ZU 62

Nov. 7, 1995
en-ro; 2653

STATEMENT

In vitro chromosome aberration assay with Uvinul TA 150 in V79 cells;
Project No.: 32M0246/934164

Assuming, that the terms "glass plate including germs" refers to the microscopic slides, I would like to comment on the request for slides of the positive and negative control groups as follows:

1. For GLP reasons microscopic slides are generally not provided externally to avoid a possible loss or damage.

2. General remarks

   - The study was carried out
     - according to internationally accepted guidelines and
     - in compliance with international GLP provisions

   - The evaluation of the various types of chromosomal aberrations is based on internationally accepted criteria, e.g.
     - Evans, H.J. and O'Riordan, M.L.; Mut. Res. 31, 135 - 148 (1975)
     - Savage, J.R.K.; J. Med. Genet. 12, 103 - 122 (1975)

Therefore, there has always been confidence in a reliable experimental performance and evaluation of our studies and thus our reports have so far been accepted worldwide by various authorities without any additional requirement.

Dr.rer.nat. G. Engelhardt
(Study Director)